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Full Length Research Paper

Occurrence of diploid and polyploid microspores in *Sorghum bicolor* (Poaceae) is the result of cytomixis

Ghaffari, S.M.

Institute of Biochemistry and Biophysics, University of Tehran, P.O.Box 13145-1384, Tehran, Iran. Tel:++98-21-61113379. Fax: ++98-21- 66404680. E-mail: ghaffari@ibb.ut.ac.ir.

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Colchicine is used in this investigation to increase chromosome doubling in *Sorghum bicolor* Line IS4546. Of a total of 500 plants which were treated, eight of them showed the occurrence of cytomixsis at meiotic stages. No polyploids were found in the treated plants. The results obtained from meiotic studies on control plants showed the 10 bivalents in majority of pollen mother cells at diakinesis and first metaphase. In eight treated plants, occurrence of cytomixis and chromosome migration were observed. Analysis of 230 pollen mother cells at first metaphase stage showed 73.91% haploid (n=10), 10.43% diploid (n=20), 7.82% triploid (n=30), 4.34% tetraploid and 3.47% pentaploid (n=50) number of chromosomes. Pollen diameters showed that the cytomictic cells differed from the normal cells. These results indicate that cytomixis can really be an effective mechanism for the production of polyploid gametes.

Key words: *Sorghum bicolor*, colchicine, cytomixis, pollen mother cells.

INTRODUCTION

Cultivated sorghum (*Sorghum bicolor* (L.) Moench.) is a crop of *Poaceae* that can be grown in harsh environments where other crops such as rice, corn grow or yield poorly (Miranda et al., 1979; FAO, 1995). This crop is an important food and feed crop in the world, and is still the principal source of energy, protein, vitamins and minerals for millions of the poorest people (FAO, 1995).

Cytoplasmic connections, is a phenomenon widely described in angiosperms (Levan, 1941; Heslop-Harrison, 1966; Risueno et al., 1969; Whelan, 1974; Sarbhoy, 1980; Peeters et al., 1985; Bione et al., 2000). The first description was made by Gates (1908), who observed delicate threads of cytoplasm connecting adjacent pollen mother cells in *Oenothera*. Gates (1911) subsequently suggested that these connections must form an important avenue of exchange between pollen mother cells (PMCs), and described the transfer of nuclear material through them from one meiocyte to another, calling the process "cytomixis". The phenomenon of cytomixis consists in the migration of chromosomes between meiocytes through cytoplasmic connections. Since cytomixis creates variation in the chromosome number of the gametes, it could be considered a mechanism of evolutionary significance. Until now cytomixis in meiocytes has been investigated in numerous species, including some grass species, but not in *S. bicolor* up till now.

This article describes the meiotic study of diploid plant of *S. bicolor* Line IS4546 (2n = 2x = 20) affected by an evident process of cytomixis during microsporogenesis.

MATERIALS AND METHODS

Colchicine is used in this investigation to increase chromosome doubling in *S. bicolor,* Line IS4546. Method of treatment used, the shoot immersion methods, is described in detail by Lesins (1955) and the conventional application of solution to the seedling shoot apex. Colchicine applications were made 3 times daily for 2 or 3 days (Table 1). Treatment commenced 4 days after planting, and 0.5, 0.2 and 0.1% colchicine drops were placed upon the shoot apex. In certain treatments, cotton swabs were wedged between the cotyledons to help prevent evaporation according to Schank and Knowles (1961).

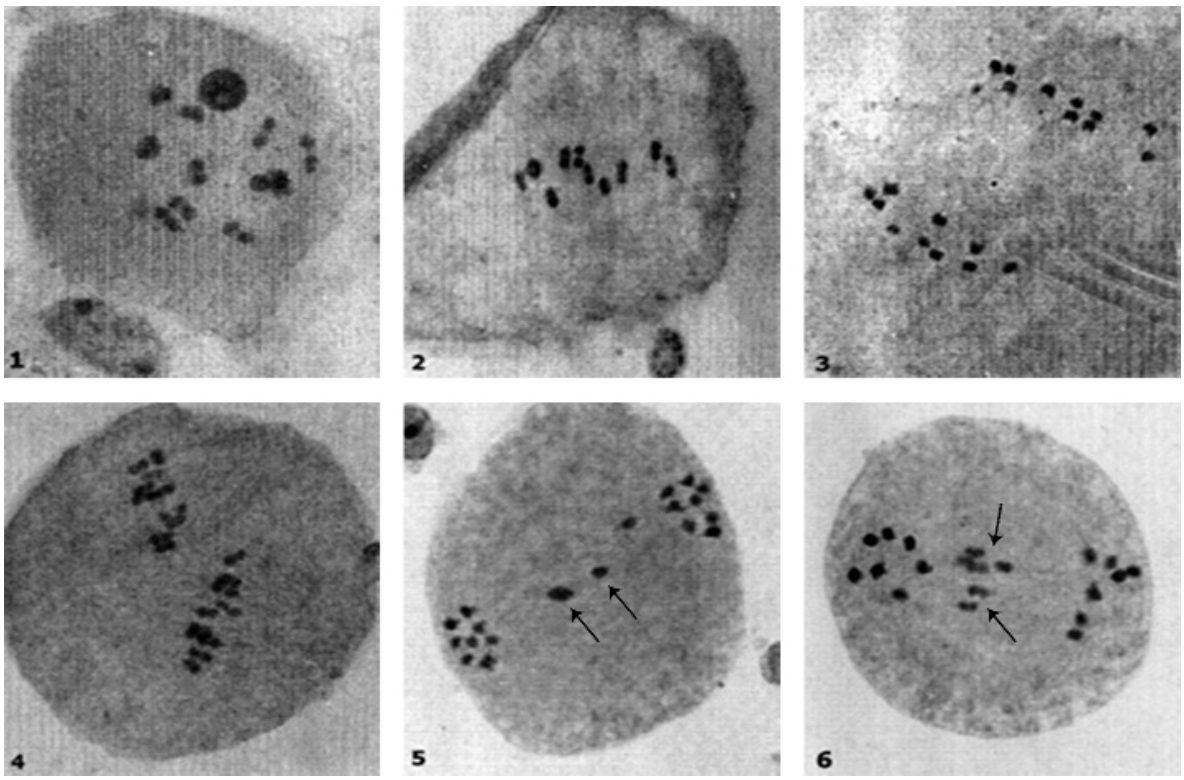
For meiotic studies on control and treated plants, flower buds at an appropriate stage of development were fixed in 96% ethanol, chloroform and propionic acid (6:3:2) for 24 h at room temperature

Table 1. Induction of cytomixsis in *S. bicolor* treated with colchicine.

Viscosity and time treated with colchicine	No. of plants treated	No. of plants that showed cytomixis	No. of plants that were tetraploids
Control	100	0	0
0.1% for 2 days	100	3	0
0.1% for 3 days	100	5	0
0.2% for 2 days	100	0	0
0.2 % for 3 days	100	0	0
0.5% for 2 days	100	0	0

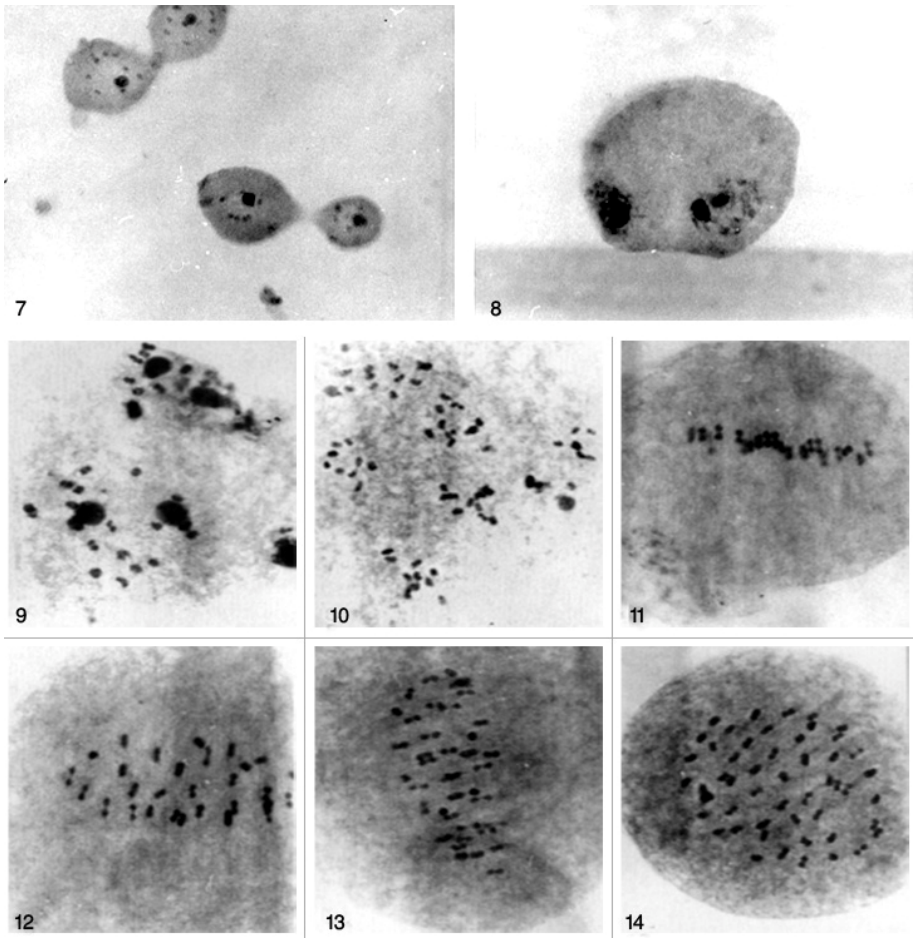
Table 2. Frequency of diploid and polyploid microspores at first metaphase.

No. of cells analyzed	Sets of chromosomes at first metaphase				
	n	2n	3n	4n	5n
170	+	-	-	-	-
24	-	+	-	-	-
18	-	-	+	-	-
10	-	-	-	+	-
8	-	-	-	-	+
%	**73.91**	**10.43**	**7.82**	**4.34**	**3.47**



Figures 1- 6: 1. Diakinesis, showing 10 bivalents; **2.** Metaphase I, n = 10; **3.** Anaphase I, showing (10 – 10) chromosome segregation; **4.** Second metaphase; **5,6.** Anaphase I, showing laggard chromosomes (arrows). X 1320.

and then stored in 70% alcohol at 4°C until used. Anthers were squashed and stained with 2% acetocarmine. All slides were made permanent by the venetian turpentine (Wilson, 1945). Photographs of chromosomes were taken on an Olympus photomicroscope at initial magnification of X330.



Figures 7 – 14. 7. Showing cytoplasmic channel between two pollen mother cells in diakinesis; **8.** Early prophase after the migration of two nuclei in the adjacent cell; **9.** Showing cytomixis of 4 PMCs in diakinesis stage; **10.** Showing cytomixis of 6 PMCs in metaphase I stage; **11.** M I, PMC with 20 II chromosomes; **12-14.** Showing triploid, tetraploid and pentaploid PMCs, respectively. X 1320.

RESULTS

Results of the field trials which involved 6 different treatments (100 plants for each treatment) are presented in Table 2. Of a total of 500 plants which were treated, eight of them showed the occurrence of cytomixsis at meiotic stages. No polyploids were found in the treated plants.

The results obtained from meiotic studies on control plants, showed the 10 bivalents in mostly of pollen mother cells at diakinesis and first metaphase (Figures 1 and 2). Also most of another stages of meiosis was regular and showed chromosome segregation (10 – 10) at anaphase I (Figure 3), and ten dyads in each pole of second metaphase (Figure 4). This results agrees with previous reports (Endrizzi, 1957; Vachova, 1978; He et al., 1997). Occasionally in some cells laggard univalents and bivalents were observed (Figures 5 and 6). On the 8 treated plants, meiotic abnormalities, included cytoplasmic connections, nuclei migration among cells were observed (Figures 7 and 8). The meiotic phases generally most affected by these abnormalities were prophase I and metaphase I, and involved with two or more mirosprocytes (Figures 9 and 10). Pollen mother cells in which metaphase I chromosomes from two, three, four and five nuclei lying close to each other were observed, but it was not clear whether they were aligned on common or adjacent spindles (Figures 11, 12, 13 and 14). Analysis of 230 pollen mother cells at first metaphase stage showed 73.91% haploid (n = 10), 10.34% diploid (n = 20), 7.82% triploid (n = 30), 4.34% tetraploid (n = 40) and 3.47% pentaploid (n = 50) respectively (Table 2). Pollen diameters showed that the polyploid microspores are different from the normal haploid cells (Table 3). It has been demonstrated that the size of pollen grains is related to the ploidy level. The presence of an evident variability in pollen size is recognized as an indication of polyploid pollen formation (Stanley and Linskens, 1974).

Table 3. Pollen diameter in poloid microspores.

No of cells analayzed	Pollen diameter (µm)				
	n	2n	3n	4n	5n
170	32.66				
24		40.11			
18			42.66		
10				46.63	
8					50.12

DISCUSSION

Cytomixis and spontaneous fusion of pollen mother cells are reported in number of angiosperm species (Levan, 1941; Heslop-Harrison, 1966; Risueno et al., 1969; Whelan, 1974; Sarbhoy, 1980; Peeters, 1985; Dagne, 1994; Bione et al., 2000; Ghanima and Talaat, 2003). Pollen mother cells deriving from the process of cytomixis can have a variable chromosome number as a consequence of the manner in which the process takes place. Meiocytes may be involved in one or more cytomictic events, and the migration of the nuclear content may involve all the chromosomes or part of the chromosomes of the donor cell. It can be expected that a greater part of the microspores deriving from such pollen mother cells will be aneuploid or polyploid.

Cytomixis has been considered by some authors as a mechanism of evolutionary importance for plants (Srivastav and Raina, 1980; Zheng et al., 1987). For other authors, it represented just an unfavorable phenomenon with deleterious effects on fertility (Marechal, 1963). Cytomixis has been investigated in numerous species (Kamra, 1960), including some grass species (Basavaiah and Murthy, 1987; Koul, 1990), but not in *Sorghum bicolor*. Investigation in *S. bicolor* confirms that the migration of the chromosomes is a real event that can not be misunderstood as an artifact produced by fixation or mechanical injuries (Gottschalk, 1970). This study also revealed some aspects that had not appeared in other cytomictic plants. For example, the first metaphase in diploid, triploid, tetraploid and pentaploid pollen mother cells indicates that the state occurs the same way as natural polyploidy plants. The results indicate that cytomixis can really be an effective mechanism for the production of polyploid microspores. Occurrence of diploid pollen mother cells has been reported in *Dactylis glomerata* subsp. *Castellata* Borrill & Parker (2n = 2x = 14) by Falistocco et al. (1995). Pollen analysis in this plant showed that at least 25% of the pollen of cytomixis plant was polyploid. These results demonstrated that in this plant cytomixis is an effective mechanism for the production of pollen grains with different ploidy levels.

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